III. 35 U.S.C. §112, Second Paragraph

Claim 1 stands rejected under the second paragraph of §112 on the grounds that adding a reagent at a concentration 0 mM is non-sensical. Claim 1 has been amended to remove step (b), which is now the subject matter of a dependent claim, claim 4. The subject matter of original claim 4 is noW the subject of two new dependent claims, claim 21 and 22.

IV. 35 U.S.C. §112, First Paragraph

Claims 1-10 are rejected under the first paragraph of §112 as lacking an enabling disclosure. Applicants respectfully traverse. The examiner has offered a number of scientific observations which are said to bear upon the enablement issue. Applicants will respond to each of these below.

First, the examiner argues that it is commonly known that "most proteins, if denatured or aggregated in an inclusion body, do not readily renature using a single method of treatment … [and that] some proteins … have no treatment that can be used to yield active protein …." While this may be true, it says nothing about the enablement of the present invention. Rather, it addresses the deficiencies in the prior art, and therefore simply highlights something the examiner already acknowledges – that the present invention is both novel and non-obvious.

Second, the examiner argues that "it is also well known in the art that the native, active form of a protein is typically not the lowest energy configuration …." This statement is false. The vast majority of proteins exist at the lowest free energy state, which is the native, biologically active species – a fact documented repeatedly in the literature. Indeed, discovery of

a kinetically-trapped, active protein that is not in a lowest free energy state is viewed as a novelty, and has been the subject of numerous papers. For example, Anfinsen, CB, "Principles that govern the folding of protein chains," *Science* 181:223-30 (1973), showed that ribonuclease spontaneously folds from the denatured state. This is the first paper known to the inventors that shows that the folding process was driven entirely by the decrease in free energy, and thus the native form of proteins appears to be the thermodynamically most stable structure. Other reports include Goldenberg & Creighton, "Energetics of protein structure and Folding," Biopolymers 24:167-182 (1985); Onuchic *et al.*, "Theory of protein folding: the energy landscape perspective," *Ann. Rev. Phys. Chem.* 48:545-600 (1997); and Shortle, D. "The denatured state (the other half of the folding equation) and its role in protein stability," *FASEB J.* 10:27-34 (1996).

The examiner is correct in noting that "the cell is actively involved in creating" the proper, low-energy configuration. However, it is incorrect to suggest that cellular processes are required because the free energy of the active state is high. Processes, such as the activity of chaperonins, are catalytic, and thus by definition act by modifying kinetics rather than altering thermodynamic energy levels. Often, during folding (*in vivo* or *in vitro*), proteins are kinetically trapped in undesirable, non-native aggregates. The present invention simply reduces the kinetic barriers to achieving the lowest free energy, active native conformation.

The examiner also argues that the application offers no guidance as to the pressure levels of either step, or the temperature range that one should employ. Applicants clearly do not understand the rejection. Even the broadest claim – claim 1 – specifies pressure ranges for both

steps (c) and (d). As for temperature, applicants are curious as to why the examiner finds this element "critical" to the present invention, as no such representation is made by applicants, and no evidence is provided by the examiner. To the contrary, the specification states that:

> The processes of the present invention can be carried out at any temperature between the freezing point of the aqueous medium (about 0°C) and the temperature at which biological activity is lost due to thermal denaturation. The upper limit will be somewhat different for each individual protein and will also be affected by the composition of the medium, pH, presence of stabilizing compounds and the like, as is known in the art. The preferred temperature for carrying about the process of the invention is within 20°C of the upper limit temperature ....

Page 7, lines 16-22. While temperature clearly can affect the claimed invention, this passage hardly indicates that a given temperature is "critical" to its success.

The examiner next complains that the Examples fail to provide evidence of "the two-step pressure disaggregation process." Applicants respectfully point out that the steps (c) and (d) actually recite overlapping ranges and, thus, the claims are generic to "one-step" and "two-step" processes. Thus, the "one step" examples do, in fact, provide enablement for the claims. However, applicants have provided the attached declaration of Dr. Ted Randolph which describes additional examples that demonstrate a "two-step" pressure treatment. See, for example, attached FIG. 5.

In addition, Dr. Randolph's declaration addresses another alleged shortcoming of the specification, which is support for refolding of "proteins" generally. As discussed, the list of proteins now shown to be susceptible to high pressure disaggregation/refolding include the dimeric protein, interferon-γ. See for example, attached FIG. 2.

In sum, applicants submit that the present application provides more than sufficient bases for finding the present claims enabled. Further, though not required, the attached declaratory submission provides additional evidence showing that one of skill in the art, using only the methods disclosed in the instant specification, could make and use the invention as now claimed. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

V. Conclusion

In light of the foreging, it is respectfully submitted that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should Examiner Guttman have any questions, he is invited to contact the undersigned attorney at (512) 536-3184.

Respectfully submitted,

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